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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:
Sharat Singh

SERIAL NO.: 09/466,369

FILED: December 15, 1999

FOR: **INDIVIDUALLY ADDRESSABLE SOLID SURFACES FOR MULTIPLEXED OPERATIONS**

EXAMINER: Sisson, B.

ART UNIT: 1655

Response

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This response is in reply to the Office Action mailed November 6, 2001 in the above-identified application.

REMARKS

Claims 1-18 are pending for prosecution in this case. Favorable consideration of the following comments relative to the outstanding rejections as they may apply to the present claims is respectfully requested for the reasons that follow.

I. Rejection Under 35 U.S.C. §103

Claims 1-18 were rejected under 35 U.S.C. §103 as being unpatentable over Still (Acc. Chem. Res. 1996, 29:155) in view of Chenchik *et al.* (U.S. Patent No. 6,087,102), Benson *et al.* (U.S. Patent No. 6,051,719), Rothman *et al.* (U.S. Patent No. 4,921,878), and Heller (U.S. Patent No. 4,824,776). This rejection is respectfully

traversed for the following reasons.

A. The Present Invention

The present invention is concerned with creating individual addresses for solid surfaces for the purpose of improving the level of multiplexing possible in a single mixture. Improving multiplexing relies upon establishing as broad a variety of labels as possible that are readily distinguished. In the claimed invention multiplexing is increased by using lanthanide dyes that yield accurate, quantitative readouts without quenching or energy transfer. When dyes with these characteristics are employed, multiplexing can be dramatically increased because both *combinations* of different dyes and their relative *proportions* can serve as variables in the address. Using both combinations and proportions as a means of increasing multiplexing, as well as the utilities and advantages of this approach to multiplex labeling, has been previously unappreciated.

Dyes that are subject to significant quenching and energy transfer are not suitable to the present invention because these reactions will reduce quantum yield and broaden emission spectra. As a result, the quantitative dynamic range of detection is reduced, and accurate measurement of differing *ratios* of dyes is not feasible. Furthermore, spectrum broadening will reduce resolution within a mixture of dyes located on a single particle. Both quenching and energy transfer prevent an accurate quantitative measurement of the composition of a dye combination, and limit multiplexing capacity to the number of individual, spectrally resolvable dyes that are available. These characteristics are factors that have limited the level of multiplexing obtained with methods making use of organic dyes.

B. The Cited Art

Still discloses the use of solid supports, or beads or particles, which are labeled with chemical moieties. These moieties are used in combinations to create labels that encode a particle associated with an individual member of a chemical library. The method as disclosed by Still is limited to qualitatively determining the presence or absence of a particular chemical moiety, and does not teach the advantages to

multiplexing by monitoring the *ratios* of chemical moieties in a label. As such, the level of multiplexing enabled by the methods of Still is limited to 2^N different labels from N different moieties (page 15 8, column 2, lines 1-3). The present invention discloses means for detecting both the combination *and ratios* of dyes composing an individual address. This dramatically increases the degree of multiplexing possible from a fixed number of different dyes. For example, with two label molecules called A and B, the methods of Still will provide for a multiplex of four (no label, A, B, I A+ I B). The level of multiplexing possible with the methods of the present invention is theoretically infinite, as the following combinations can be distinguished: 1A, 1B, 1A+1B, IA+2Bs, IA+3Bs, *etc*, 2As+1B, 3As+ 1B, etc.

Chenchik *et al.* discloses arrays of polymeric targets on a rigid support, where the polymeric targets of the arrays are arranged according to size. The targets are contacted with labeled probes to bind the probes to the targets. The hybridization pattern of the labeled probes is then detected. The position in the pattern to which the probe binds and where the probe is subsequently detected yield information regarding the size of the target molecule. Chenchik *et al.* discloses a list of fluorophores used in the described assay format (column 9, lines 25-65). The Examiner cites this list in light of Applicant's disclosure, however it includes only one class of labels that is appropriate to the present invention (that of the lanthanide chelates), along with several others that are not useful in the present invention, including fluorescein, rhodamine, and thizole orange-ethidium heterodimer (all dyes that are subject to both the quenching and energy transfer reactions discussed above). Chenchik *et al.* do not teach the advantages or particular properties of the dyes they list, and therefore their listing cannot be construed as a suggestion for the use of lanthanide dyes in particular. Furthermore, Chenchik's disclosure does not suggest improved means of multiplexing. In the present invention, lanthanide dyes are employed for overcoming severe multiplexing limitations encountered with the other types of dyes listed by Chenchik *et al.*

There are additional characteristics of lanthanide dyes (as compared to organic dyes) that confer previously unappreciated advantages for quantitative, multiplexed

labeling. These dyes absorb within a narrow spectrum, therefore a larger multiplex can be monitored using a single excitation laser. Lanthanide dyes emit over a broader range of frequencies and have very sharp emission lines, which facilitate resolution of multiple dyes within a mixture. Furthermore, the present invention discloses methods for labeling particles by dyeing them, which confers much greater flexibility in controlling dye combinations and ratios than can be obtained by chemical reaction.

Benson *et al.* discloses the use of multiple color fluorophores in the context of an automated DNA sequencing method, wherein each of four dyes used represents a specific nucleotide base. This reaction is conducted as a multiplex to generate sequence information for all four nucleotides in a single sample. DNA sequence information is obtained by generating a ladder of bands in a sequencing gel, each band yielding a fluorescent signal from the single dye that terminates chain elongation at that position in the sequence. While the sequencing reaction is effectively a multiplex of four individual reactions, the fluorescent signals generated from each band in the gel arise from a *single* fluorescent species, and as such, the method as disclosed provides for a very restricted level of multiplexing. The advantages of using dyes in combination on a single entity are not taught. The sequencing of *one* template from individual dye signals can be achieved because the required set of four organic dyes with resolvable emission spectra is available. However, a multiplex of eight, required for sequencing two nucleic acid templates, is not currently possible using organic dyes. The methods disclosed in the present invention would allow monitoring many separate sequencing reactions in a single mixture.

Rothman *et al.* discloses a list of fluorophores used in a variety of assay formats (column 10, lines 25-32). However, as pointed out with respect to the Chenchik *et al.* citation, all but the lanthanide dyes disclosed by Rothman are not useful in the present invention, and this citation should not serve as prior art simply in light of Applicant's disclosure. The organic dyes of Rothman *et al.* are subject to both the quenching and energy transfer reactions discussed above.

Heller discloses the use of a variety of fluorescent compounds, including lanthanide complexes, as useful in his invention (column 5, lines 25-27). Heller's list includes radioactive, colorimetric, and fluorescent labels, including fluorescein, Texas Red, Lucifer Yellow, pyrene, lanthanide complexes, etc. (column 5, lines 16-27). However, as pointed out with respect to the Chenchik *et al.* citation, all but the lanthanide dyes disclosed by Heller are not useful in the present invention, and this citation should not serve as prior art simply in light of Applicant's disclosure.

Heller's invention is concerned with improving the sensitivity of hybridization assays, and he suggests the possible use of more than one fluorophore per probe *in order to improve detection* (column 5, lines 29-33). Specific methods for improving detection are not taught, so it is not possible to know the meaning of this statement. Several interpretations are possible, including increasing signal strength by attaching multiple molecules of a single type of dye to each probe, creating a unique signal through a Stokes shift by attachment of a pair of dyes that allow energy transfer, or differentiating species in a multiplex by using dye combinations. Heller does not specify conditions or dye combinations. His disclosure does not suggest a multiplexed system of quantitatively differentiated dye concentrations, nor does it reasonably suggest the use of multiple dyes in combinations in a single particle. The use of and advantages resulting from quantitatively measuring a combination of fluorophores present in different ratios within individual particles are not taught.

C. Analysis

According to MPEP § 2143, three basic criteria must be met to establish a case of obviousness. "First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the references or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations." MPEP § 2143.

None of the references, taken singly or in combination, provides motivation for combining the references along the lines of the invention. Obviousness requires some

logical reason for combining the references at hand; otherwise, the use of the references will entail prohibited hindsight (e.g., *In re Semaker*, 217 USPQ 1). In particular, the fact that references can be combined does not make the combination obvious unless the prior art also contains something to suggest the desirability of that combination.

In the present case, the cited references do not show or suggest the critical elements of the invention, for the reasons discussed above.

Even if these elements were disclosed in the cited references, the prior art does not recognize the advantages of the invention, and thus provides no motivation for combining elements along the lines of the invention.

Regarding the listing of lanthanide chelates as one possible dye for the inventions of Chenchik *et al.*, Rothman *et al.*, and Heller, the test which determines whether an element of an invention has been anticipated by a reference is whether the description of the invention in the reference is "sufficient to put the public in possession of the invention." *In re Arkely*, 455 F.2d 586, 172 USPQ 524 (CCPA 1972); *In re Schaumann*, 197 USPQ 5, 10 (CCPA 1978). In the present case, the issue is whether the disclosures of Chenchik *et al.*, Rothman *et al.* and Heller describe the claimed invention with sufficient specificity to put the public in possession of the invention.

Applicants submit that Chenchik *et al.*, Rothman *et al.* and Heller fails to meet this legal standard and does not disclose the claimed invention with sufficient specificity to put the public in possession of the invention. As discussed above, the laundry list of dyes recited in each of these references includes numerous dyes known to be ineffective in the present invention.

Given that a number of dyes are listed in Chenchik *et al.*, Rothman *et al.* and Heller that are not useful in the present invention because they are subject to both the quenching and energy transfer reactions discussed above, Applicant submits that the invention as presently claimed is not disclosed by Chenchik *et al.*, Rothman *et al.* and

The mere naming of a compound in a reference naming a myriad of compounds, without more, cannot constitute a description of the compound. *In re Wiggins*, 488 F.2d 538, 179 USPQ 421 (CCPA 1973). In *Wiggins*, claims to a compound useful for treating Parkinson's disease were rejected as anticipated by a reference that disclosed the claimed compound but that failed to describe a synthetic method suitable for

preparing the compound. A method for preparing the compounds was not developed until a date later than that of the reference.

In reversing the Examiner's rejection of the claims, the court held that a reference's listing of specific compounds within the scope of the claimed compound constituted nothing more than mere speculation about their potential or theoretical existence and, hence, was not a description of the compounds within the meaning of §102. *Id. at* 543, 179 USPQ 421, 425.

Similarly, in the present case, Chenchik *et al.*, Rothman *et al.* and Heller list dozens of dyes. Many of the dyes listed are known to be ineffective for the multiplexed determination of the invention. It cannot be known which, if any, of the listed compounds might be effective, since the mere listing of the compounds is nothing more that speculation about their usefulness. Thus, the speculative disclosure of Chenchik *et al.*, Rothman *et al.* and Heller of lanthanide dyes as one of dozens of possible candidates cannot be said to place the public in possession of the claimed invention.

Dependent claims 2-6 incorporate all the subject matter of claim 1 and add additional subject matter, which makes them *a fortiori* and independently patentable over Still, Chenchik *et al.*, Benson *et al.*, Rothman *et al.*, and Heller. Similarly, dependent claim 8 in relation to claim 7, and 11-18 in relation to claim 10 are *a fortiori* and independently patentable over Still, Chenchik *et al.*, Benson *et al.*, Rothman *et al.*, and Heller.

Accordingly, Applicant respectfully requests withdrawal of the rejection under 35 U.S.C. §103.

II. Conclusion

In view of the above remarks, the applicants submit that the claims now pending are in condition for allowance. A Notice of Allowance is, therefore, respectfully requested.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 838-4405.

Respectfully submitted,

Date: 5-6-02

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